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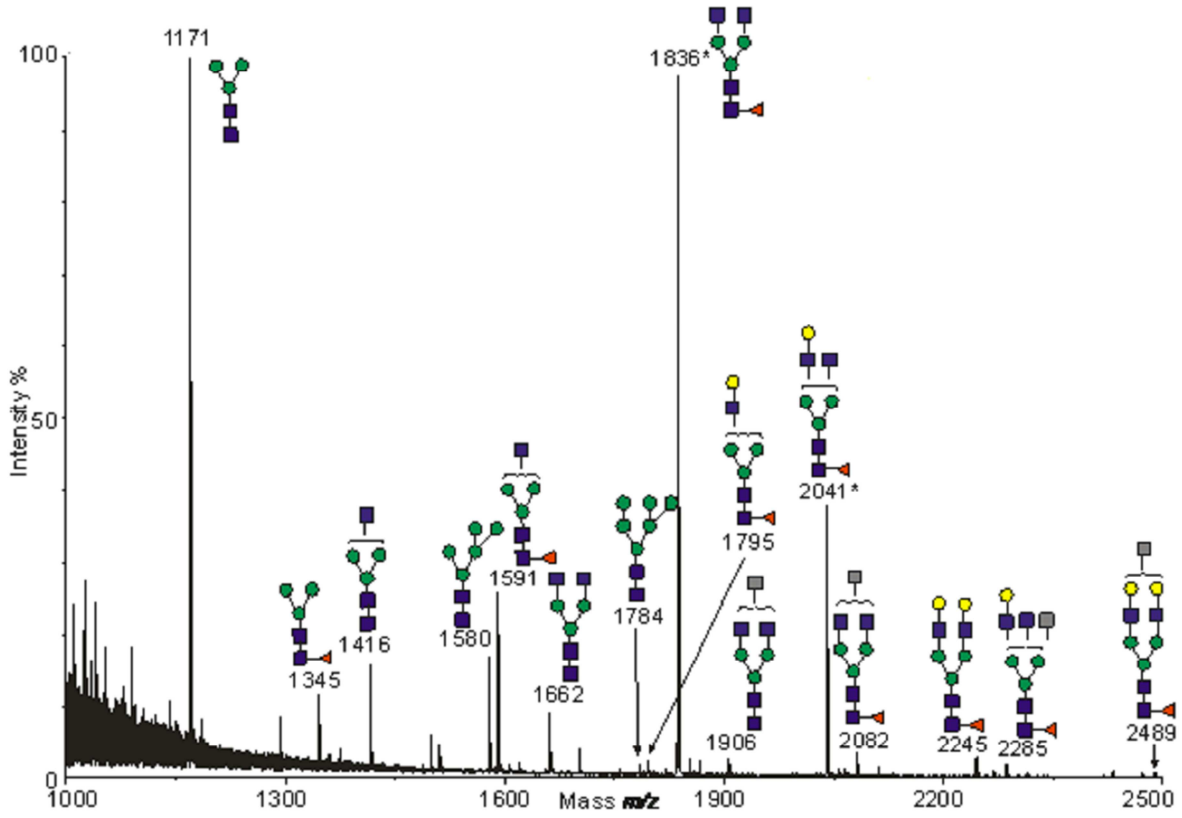

Characterization of the *N*-glycans of female *Angiostrongylus cantonensis* worms

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ABSTRACT

Glycoconjugates play a crucial role in the host-parasite relationships of helminthic infections, including angiostrongyliasis. It has previously been shown that the antigenicity of proteins from female *Angiostrongylus cantonensis* worms may depend on their associated glycan moieties. Here, an *N*-glycan profile of *A. cantonensis* is reported. A total soluble extract (TE) was prepared from female *A. cantonensis* worms and was tested by western blot before and after glycan oxidation or *N*- and *O*-glycosidase treatment. The importance of *N*-glycans for the immunogenicity of *A. cantonensis* was demonstrated when deglycosylation of the TE with PNGase F completely abrogated IgG recognition. The TE was also fractionated using various lectin columns [*Ulex europaeus* (UEA), c*oncanavalin* A (Con A), *Arachis hypogaea* (PNA)*, Triticum vulgaris* (WGA) and *Lycopersicon esculentum* (LEA)], and then each fraction was digested with PNGase F. Released *N*-glycans were analyzed with matrix-assisted laser desorption ionization (MALDI)-time-of-flight (TOF)-mass spectrometry (MS) and MALDI-TOF/TOF-MS/MS. Complex-type, high mannose, and truncated glycan structures were identified in all five fractions. Sequential MALDI-TOF-TOF analysis of the major MS peaks identified complex-type structures, with a α1-6 fucosylated core and truncated antennas. Glycoproteins in the TE were labeled with BodipyAF558-SE dye for a lectin microarray analysis. Fluorescent images were analyzed with ProScanArray imaging software followed by statistical analysis. A total of 29 lectins showed positive binding to the TE. Of these, *Bandeiraea simplicifolia* (BS-I), PNA, and *Wisteria floribunda* (WFA), which recognize galactose (Gal) and *N*-acetylgalactosamine (GalNAc), exhibited high

affinity binding. Taken together, our findings demonstrate that female *A. cantonensis* worms have characteristic helminth *N*-glycans.

KEYWORDS: *Angiostrongylus cantonensis*, Glycans, Lectin array, Mass spectrometry

1. INTRODUCTION

Eosinophilic meningoencephalitis (EoM) is caused mainly by the nematode *Angiostrongylus cantonensis,* referred also as cerebral angiostrongyliasis (CA), which is endemic in many parts of the world, particularly in tropical and subtropical countries (Morassutti et al., 2014; Wang et al., 2012, 2008).

Humans may acquire the infection by consuming third stage larvae (L3) that are present in contaminated water, in raw and undercooked food, fruits, and vegetables, or in mollusks, which is the intermediate host for this parasite (Wang et al., 2008). After ingestion, the larvae access the central nervous system via bloodstream and cause severe inflammation resulting in EoM, which in some cases may be fatal (Graeff-Teixeira et al., 2009; Wang et al., 2008).

Female *A. cantonensis* worms express different proteins that may influence many physiological and pathological processes, including interactions with the host's immune system (Song et al., 2012; Wang et al., 2008). For example, when an antigen prepared from the reproductive tubes of female *A. costaricensis* worms, a species which can cause abdominal angiostrongyliasis, high antigenicity was observed with human sera (Bender et al., 2003). In addition, the main diagnostic antigen for CA is a glycoprotein that is obtained from female *A. cantonensis* worms (Morassutti et al., 2012).

Several studies have highlighted the importance of glycans as dominant antigens based on their ability to interact with a host's immune system, mainly because these glycan are located on the surface of the parasite (Nyame et al.,

2004). Especially, glycans can induce humoral responses (Nyame et al., 2003; Sher et al., 2003; van Die & Cummings, 2006; Van Liempt et al., 2007).

In a recent proteomic study of the 31-kDa antigen from *A. cantonensis*, recognition of this antigen by human antibodies was found to be dependent on the carbohydrate moieties present (Morassutti et al., 2012). Correspondingly, attempts to produce recombinant antigens of *Angiostrongylus* in prokaryotic systems have failed (Morassutti et al., 2013), potentially due to a lack of proper glycosylation (Dell et al., 2010).

This study aimed to described the glycan repertoire of *A. cantonensis* female worms, and investigate its importance for the immune recognition.

2. MATERIALS AND METHODS

2.1. Worms and antigen preparation

The life cycle of *A. cantonensis* worms have been maintained at the Laboratório de Biologia Parasitária (PUCRS, Porto Alegre, Brazil) since 2013. *Wistar* rats are used as definitive hosts and *Biomphalaria glabrata* serve as intermediate hosts. Briefly, *Wistar* rats are infected with 104 larvae by gavage inoculation. Forty-two days post-infection, the rats are sacrificed and the worms are collected and frozen at -20 ℃. Animal handling for this study was performed according to the guidelines of our institute and the experimental protocol was approved by the PUCRS Ethics Committee for Animal Use (no. 13/000331).

In this study we used TE of the female *A. cantonensis* worms as the main antigen used for immune diagnosis of CA, 31-kDa compound, derives from this extract (Morassutti et al., 2014). A total soluble extract (TE) was obtained from harvested female *A. cantonensis* worms that were macerated in liquid nitrogen

and homogenized in an extraction buffer [phosphate-buffered saline (PBS; pH 7.4), 0.01% Triton X- 100, and freshly added protease inhibitors (Qiagen). The suspension was centrifuged at 12,000 x *g* for 1 h at 4 ºC. Total protein concentrations of the TE supernatants were estimated with the Qubit kit (Invitrogen, Mount Waverly, Victoria).

2.2. Western blot (WB) with glycosidases

TE (100 ng/line) were resolved in 12% SDS-PAGE gels and immediately electro-transferred into nitrocellulose membranes. These membranes were then blocked with 5% milk for 2 h at room temperature (RT) and incubated for 2 h with either individual or a pool of three individual sera of positive samples of CA (1:200 dilution). The membranes were washed three times and probed with a secondary peroxidase-conjugated anti-human IgG (diluted 1:2000; Abcam, Cambridge, UK) for 2 h at RT. Diaminobenzidine (DAB) (Sigma-Aldrich, St. Louis, MO, USA; 0.05% DAB and 0.015% H2O2 in PBS, pH 7.4) was added to visualize the bound antibodies.

2.2.1. N-Glycosidase F (PNGase F) treatment

PNGase F treatment was performed according to the manufacturer's instructions (recombinant PNGase F, 500,000 U/mL; BioLabs, UK). Briefly, PNGase F was mixed with the TE at a final concentration of 500 U/µg total protein and the samples were incubated overnight at 37 ℃. PBS was used as a negative control. The recognition by CA-positive or negative sera was tested by WB (described above).

2.2.2. O-Glycosidase treatment

TE (1 µg) was either incubated with α-L-fucosidase (0.2 U; Sigma-Aldrich) for 18 h and then were incubated with *O*-glycosidase (0.02 U; Sigma-Aldrich) for another 18 h at RT, or were directly incubated with *O*-glycosidase without pre-treatment with α-L-fucosidase. After, TE (100 µg/line) was resolved in 12% SDS-PAGE gel, and the recognition by CA-positive or negative sera was tested by WB (described above).

2.2.3. Sodium meta-periodate treatment

TE (100 ng/line) were resolved in 12% SDS-PAGE gels and immediately electro-transferred into nitrocellulose membranes. The membranes were washed three times with PBSTween 0.05% (v/v). The membranes were incubated for 30 min with 100 mM NaOAc (pH 5.0), then were incubated with a sodium *m*-periodate solution (20 mM NaIO4 diluted in 100 mM NaOAc), and kept at 37 ℃ in the dark. After 1 h, the membranes were washed with 100 mM NaOAc and were incubated with 50 mM NaBH4 in PBS-Tween 0.05% (v/v) for 30 min at RT. WB were then performed (described above).

2.3. Lectin columns

To enrich the glycoconjugate forms of the *A. cantonensis* antigen, well known commercially available biotinylated lectins were chosen to bind glycan moieties of both *N*- and *O*- glycans: mannose, fucose, galactose, *N*-acetylglucosamine*, and* chitobiose core: *Concanavalin* A (Con A), *Ulex europaeus* (UEA)*, Arachis hypogaea* (PNA), *Lycopersicon esculentum* (LEA), and *Triticum vulgaris* (WGA) (Sigma-Aldrich). Briefly, 200 µL of streptavidin-

coated magnetic beads were mixed with 50 µg of each biotinylated lectin in separate microtubes, and these tubes were incubated overnight at RT. The supernatant from each tube was subsequently removed and the columns were washed five times with Tris-buffered saline (TBS, pH 7.4). TE (300 µg) was then added to each column and the tubes were incubated at RT for 1 h. Unbound molecules were subsequently removed with aspiration of the supernatant and five washes with TBS. Glycans and glycoconjugated molecules bound to the columns were released when 50 µL of 0.1 M sodium borate (pH 6.5) was added to each tube. The resulting fractions were stored at -20 ℃ until MS analysis.

2.4. Mass spectrometry (MS) analysis

The collected fractions of purified glycoconjugates were reduced, carboxymethylated, and digested with trypsin for 5h at 37℃ in 50 mM ammonium bicarbonate buffer (pH 8.4). *N*-glycans were released from the purified glycopeptides by PNGase F digestion, which was performed with 0.6 units of PNGase F for 16h at 37℃ in 50 mM ammonium bicarbonate buffer (pH 8.4). The *N*-glycans were derivatized by permethylation. *N*-glycan profiles were acquired with a matrix-assisted laser desorption ionization-time-of-flight (MALDI-TOF) mass spectrometer (Voyager-DETM STR PerSeptive Biosystems). Permethylated samples were dissolved in 10 µL of methanol, then 0.5 µL of each of these samples were added to 0.5 µL of the matrix (20 mg/ml of 2,5-dihydrobenzoic acid in 70% (v/v) aqueous methanol). The sample/matrix mixture was loaded onto a sample plate for MS analysis. MALDI-TOF/TOF experiments were performed with a 4800 Proteomics Analyzer (Applied Biosystems) using 3,4-diaminobenzophenone (20 mg/ml in 70% (v/v) aqueous

acetonitrile) as the matrix. Both instruments were operated in the reflectron positive mode (North et al., 2010).

2.5. Lectin array

The glycoproteins present in the *A. cantonensis* TE were labeled with 10 mM BodipyAF558 succinimidyl ester dye (Invitrogen) in PBS (pH 7.4) for 45 min at RT, the excess dye removed and then were applied to a lectin array according as described in Ardnt et al (2011). The lectin array slides were printed with 86 lectins, in three different concentrations (500, 250 and 125 µg/mL). BSA was used as a negative control. The acquisition of fluorescent images and subsequent measurements and analyses were made with a ProScanArray Microarray scanner (Perkin Elmer, Waltham, MA) and ScanArray Express software (Perkin Elmer). The mean and standard deviation value were determined in triplicates. *T*-tests were performed using Microsoft EXCEL software to analyze the statistical significance of the data. CVs were calculated to assess reproducibility.

In this study, we considerate that the lectins bind with low affinity when positive bind was observed just in spots containing 500 µg/mL of lectin; moderate affinity, when the bind was positive also in spots with 250 µg/mL of lectin; and high affinity when positive bind was observed in spots containing 125 µg/mL of lectin.

3. RESULTS

3.1. TE from female *A. cantonensis* worms contains *N*-glycans, which are essential for immune recognition

TE that was prepared from female *A. cantonensis* worms was enzymatically or chemically treated to remove or modify glycans with *N*-glycosidase (PNGase F), *O*-glycosidase, or *O*-glycosidase associated with α-L-fucosidase or *m*-periodate. Recognition of the antigen by CA-positive sera was abrogated when the TE was *N*-deglycosylated (Figure 1). The same results were observed when *m*-periodate was used for glycan oxidation.

3.2. A structural analysis of *N*-glycans from female *A. cantonensis* worms

TE prepared from female *A. cantonensis* worms was also fractionated with five biotinylated lectins: Con A, PNA, WGA, UEA, and LEA. Each fraction was analyzed independently by MS (Table 1, Figure 2). MALDI-TOF-MS data showed that fractions from all five columns presented a very similar set of *N*-glycans, including complex-type (Fuc0–1Hex3–5HexNAc3–5), high mannose (Hex5–7HexNAc2), and truncated structures (Fuc0–1Hex3HexNAc2). However, there were some variations in the fractions as well. For example, a high mannose glycan with *m/z* at 1988 was only detected in the fraction from the UEA column, while the signal at *m/z* 1345, representing a truncated structure of Fuc1Man3GlcNAc2, was absent in the same UEA column sample. In addition, only a few structures were observed in the samples from the LEA column (Table 1, Figure 2).

The MS spectra of all the samples were dominated by signals at *m/z* 1836 and *m/z* 2041. Based on sequential MALDI-TOF-TOF analyses that were subsequently performed (Figure 2), as well as MS/MS fragmentation data and

available knowledge regarding biosynthetic pathways, we propose that the corresponding structures for these two signals are core fucosylated (α1-6) biantennary glycans, the structure at m/z 1836 has two unextended GlcNAc antenna, whilst the structure at m/z 2014 has one unextended GlcNAc antenna and one Gal-GlcNAc antenna.

3.3. Lectin binding profile of female *A. cantonensis* glycoproteins

A panel of 86 lectins at three different concentrations was used to assay the glycan signature of the TE obtained from female *A. cantonensis* worms. Table 2 summarizes all of the lectins that exhibited positive binding, along with, their respective degree of affinity and the glycan structure which is known to interact with each lectin (Tao et al., 2008). This analysis demonstrated that glycoproteins present in the TE mainly bound with high affinity to *Bandeiraea simplicifolia* (BS-I), *Arachis hypogaea* (PNA), and *Wisteria floribunda* (WFA) lectins. Conversely, the TE bound with less affinity to lectins that have been shown to bind structures such as fucose (*Pseudomonas aeruginosa* lectin - LecB (PA-IIL), fucosylated core (*Lens culinaris* - LcH), complex-type structures such as Galβ1-4GlcNAcβ1-2Man (*Phaseolus vulgaris* - PHA-L), and mannose-terminal structures (*Galanthus nivalis* - GNA).

4. DISCUSSION

To our knowledge, this study presents the first data for glycans from *A. cantonensis* worms. The diversity of the *N*-linked glycans that were detected was demonstrated with MS and lectin microarrays. Also we have shown that

antigenicity of the TE was dependent on the presence of carbohydrate structures.

Previously, by used carbohydrate oxidation, our group demonstrated that glycans are essential for an immunodiagnosis of angiostrongyliasis (Morassutti et al., 2012). In the current study, we further demonstrate the importance of glycans for immunoglobulin recognition, and also show that this recognition is specifically due to *N*-glycans, since total abrogation of sera recognition was observed after the TE was subjected to an *N*-glycosidase treatment. In contrast, when an *O*-glycosidase treatment was applied to the TE, no change in IgG recognition was observed. However, it should be noted that *O*-glycosidase has a relatively restricted activity, just only against the Core 1 disaccharides (Fijita et al., 2005) (Figure 1).

The present data is in accordance with the results of several studies which have demonstrated that immunodominant epitopes of nematode surfaces, or excreted/secreted antigens, are often *m*-periodate and/or peptide-*N*-glycosidase sensitive (Haslam et al., 2003; Hokke et al., 2007; Okano et al., 2001; Sotillo et al., 2014; Talabnin et al., 2013). However, there are some exceptions where *O*-glycans have been found to be more immunogenic (Sotillo et al., 2014).

The MALDI-TOF spectra of the *N*-glycans present in the TE prepared from female *A. cantonensis* worms (Table 1), that were enriched using five different lectin columns, trapped a very similar set of *N*-glycans in each fraction. These results suggest that the glycoconjugates present in the TE may have more than one site of glycosylation that can be occupied by different glycan structures.

The characteristic features that have been observed for glycans from other helminths include termination with Gal and GlcNAc (Haslam et al., 1996, 1998, 2003; Hokke et al., 2007; Jang-Lee et al., 2005; Wisnewski et al., 1993; Wuhrer et al., 2006) and an absence of sialic acid, as it is believed that this sugar in these parasites (van Die & Cummings, 2010). Nevertheless, among helminths, sialic acid has been documented in *Mesocestoides vogae* (Medeiros et al., 2008) and *Echinococcus granulosus* (Alvarez-Errico et al., 2001), and in both cases, it was not detected by structural analyses, but rather by immune affinity assays with lectins and monoclonal antibodies.

The lectin microarray analysis performed in the present study showed evidence of sialic acid present in the glycoproteins of female *A. cantonensis*, since positive binding was observed to *Limax flavus* (LFA), *Limulus polyphemus* (LPA), and *Polyporus squamosus* (PSL) lectins, which have previously exhibited specificity for sialic acid (Tao et al., 2008). It is important to note that LFA is similar to LPA with respect to its ability to bind different forms of sialic acid, although LPA may also react with GlcNAc and glucuronic acid. These observations partly explain the present results, yet they do not clarify the LFA binding that was observed (Table 2).

In addition, the fact that sialic acid was observed only by the lectin array analysis and not MS experiments may be due to the five lectins chosen for TE enrichment did not include any lectin able to bind the glycan structure of sialic acid. However, false positive binding in the lectin-based analyses is also possible. Therefore, additional studies that include the LFA, LPA, and PSL columns, as well as MS and a molecular analysis for the identification of specific

sequences for enzymes involved in sialic acid synthesis, are necessary to confirm the present data.

Compared with the characteristics of the glycans found in other helminths, it is notable that the Lewis X epitope and multi-fucosylated terminal antennae were not detected in the present study. Fucose was detected at the proximal GlcNAc of the chitobiose core of the *N*-glycans (Fucα1-6GlcNAc) of the TE after digestion with PNGase F. Subsequent digestion of the enriched fractions with PNGase A and an MS analysis did not further identify any glycan structures (data not shown). This is consistent with the observation that PNGase F digestion resulted in the complete abrogation of immune recognition by the female *A. cantonensis* antigen (Figure 1), indicating that the glycans which have immunodiagnostic importance have a core containing fucα1-6, and this is commonly observed among helminths (Haslam et al., 1996, 1997, 1998; Hokke et al., 2007; Houston and Harnett, 2004).

The MS signal at *m/z* 2104 contains a terminal Gal-GlcNAc. Based on MS/MS fragmentation and currently available knowledge regarding biosynthetic pathways, it is likely that this terminal glycan is Galβ1-4GlcNAc (LacNAc). Studies that have compared the role of LacNAc and the fucosylated homologous (Lewis X) to the shifting of a host immune response towards a Th-2 type have concluded that the fucosylated structure alone is able to drive the immune response towards Th-2 (Okano et al., 2001; Thomas et al., 2003). The ability of parasites to shift a host's response is known to be an important strategy for parasite survival (Sher et al., 2003). In addition, the fact that the glycans from female *A. cantonensis* worms did not contain fucosylated terminal antennae suggest that further studies of the role of glycans during the infection

of permissive versus non-permissive hosts would provide a better understanding of parasite biology and host-parasite relationship.

Of the 86 lectins that were included in the microarray used, 29 showed positive binding to the female *A. cantonensis* TE (Table 2). Of these, three were associated with high affinity interactions and 18 were associated with medium affinity interactions. Interestingly, however, three of the lectins that were used for column purification, UEA, WGA, and LEA, did not exhibit positive binding in the lectin array experiments (Table 1). It is possible that either the glycans present in the TE have very low affinities for these lectins, or the lectins were degraded in the array (Tao et al., 2008). In general, glycoproteins from female *A. cantonensis* worms have been found to bind BS-I, PNA, and WFA lectins, and these are lectins that recognize oligosaccharides such as Gal and GalNAc (terminal). Fucose and complex-type structures were also among structures recognized in the lectin array assay of the present study, and these results are consistent with the glycans that were identified in the MS analyses conducted in the present study and in previous studies with *Schistosoma* parasites (Xu et al., 1994).

N-linked glycans are major targets for the host immune system as they play a vital role in immunomodulation by inducing Th-2 type responses (Nyame et al., 2002; Pearce et al., 2004). Thus, *N*-linked glycans may be key to successful parasite defense strategies. The infection of humans by *A. cantonensis* is associated with an exacerbated eosinophilic response that causes inflammatory lesions (Graeff-Teixeira et al., 2009). The structural diversity of glycans, coupled with their accessibility, support a central role for glycans in regulating parasite interactions. Thus, immune and structural

characterizations of glycans from *A. cantonensis* with highly specific and sensitive methodologies may facilitate a diagnosis of CA based on the detection of anti-glycan antibodies. Correspondingly, the anti-tyvelose-containing structure of *Trichinella spiralis* (Ellis et al., 1997) and anti-Lewis X antibodies, as well as LDN and the LDNF of *Schistosoma* (Nyame et al., 2003), have been considered potential targets for the immunodiagnosis of parasite diseases.

Based on the ability of lectin-based analyses to discriminate glycan isomers, this approach has the potential to provide a detailed profile of glycoproteins present in different extracts, especially in combination with other techniques. To our knowledge, the present study represents the first report of glycan data for *A. cantonensis*. Furthermore, we have demonstrated that female *A. cantonensis* worms have mainly complex-type *N*-glycans, with core α1-6 fucosylated and truncated antennas, and these are similar to those observed in other helminths. These insights are important for improving our understanding of host-parasite interactions, and especially for our understanding of the pathogenesis of angiostrongyliasis.

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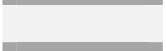
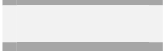
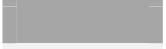
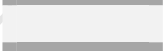
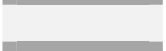
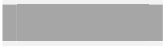
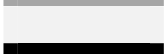
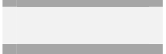
Table 1. Assignments of molecular ions ($[M+Na]^+$) observed in MALDI-TOF spectrum of permethylated *N*-glycans in the TE prepared from female *A. cantonensis* worms.

Proposed Structure	Signal *m/z*	Glycan type	Lectin columns
$Man_3GlcNAc_2$	1171	Truncated	UEA, ConA, PNA, WGA
$Fuc_1Man_3GlcNAc_2$	1345	Truncated	ConA, PNA, WGA
$Man_3GlcNAc_3$	1416	Complex	UEA, ConA, PNA, WGA
$Man_5GlcNAc_2$	1580	High mannose	UEA, ConA, PNA, WGA, LEA
$Fuc_1Man_3GlcNAc_3$	1591	Complex	UEA, ConA, PNA, WGA, LEA
$HexNAc_2Man_3GlcNAc_2$	1662	Complex	UEA, ConA, PNA, WGA
$Man_6GlcNAc_2$	1784	High mannose	UEA, ConA, PNA, WGA
$Fuc_1Hex_1Man_3GlcNAc_3$	1795	Complex	UEA, ConA, PNA, WGA
$Fuc_1HexNAc_2Man_3GlcNAc_2$	1836	Complex	UEA, ConA, PNA, WGA, LEA
$HexNAc_3Man_3GlcNAc_2$	1906	Complex	UEA, ConA, PNA, WGA
$Man_7GlcNAc_2$	1988	High mannose	UEA
$Fuc_1Hex_1HexNAc_2Man_3GlcNAc_2$	2041	Complex	UEA, ConA, PNA, WGA, LEA
$Fuc_1HexNAc_3Man_3GlcNAc_2$	2082	Complex	UEA, ConA, PNA, WGA
$Fuc_1Hex_2HexNAc_2Man_3GlcNAc_2$	2245	Complex	UEA, ConA, PNA, WGA
$Fuc_1Hex_1HexNAc_3Man_3GlcNAc_2$	2285	Complex	UEA, ConA, PNA, WGA
$Fuc_1Hex_2HexNAc_3Man_3GlcNAc_2$	2489	Complex	UEA, ConA, PNA, WGA

UEA: *Ulex europaeus;* ConA: *Concanavalin* A; PNA: *Arachis hypogaea;* WGA: *Triticum vulgaris;* LEA: *Lycopersicon esculentum* (Tomato).

Table 2. Lectin binding signature of the TE prepared from female *A. cantonensis* worms.

Lectin	Affinity to	Binding intensity
AAA	Fucα$_{1-2}$	
ACA	Galβ$_{1-3}$GalNAc	
BDA	GalNAcα, GalNAcβ	
BPA	GalNAcα, Galα	
BS-I	Galα, GalNAcα (terminal)	
CA	Galβ$_{1-4}$GlcNAc, GalNAcβ$_{1-4}$GlcNAc	
ConA	Man (Terminal, Branched), GlcNAc	
CPA	Manα? Man?	
CSA	GalNAcα (Terminal)	
ECA	Galβ$_{1-4}$GlcNAc (Terminal)	
EEA	GalNAcβ	
GNA	Manα (Terminal)	
GS-I-B4	Galα (Terminal)	
HPA	GalNAcα (Terminal)	
LcH	Man/GlcNAc core with Fucα$_{1-6}$	
LFA	Neu5Ac	
LPA	Neu5Ac	
LBA	GalNAcα, GalNAcα$_{1-3}$(Fucα$_{1-2}$)Gal	
MNA-G	Galα, Galβ	
PEA	Manα, Glcα, GlcNAcα	
PHA-L	Galβ$_{1-4}$GlcNAcβ$_{1-2}$Man	
PHA-M	N/A	
PHA-P	N/A	
PNA	Galβ (Terminal)	
PSL	Neu5Acα$_{2-6}$Galβ$_{1-4}$GlcNAc,	
SJL	Galα	
TKA	Galβ, Galβ$_{1-4}$Glc (Lactose)	
WFA	GalNAcα, GalNAcβ	
LecB (PA-IIL)	Fuc	

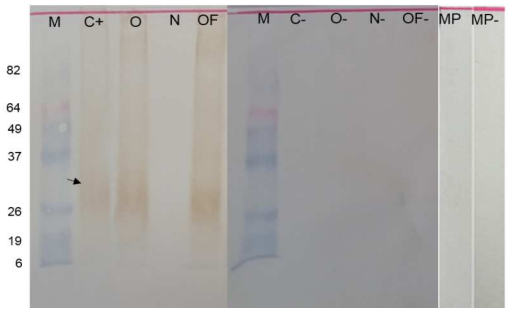
Dark: High affinity; Grey: moderate affinity; White: low affinity.

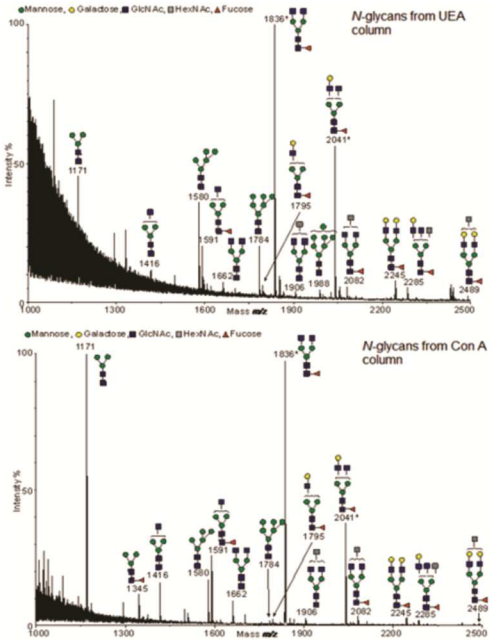
Figure Legends

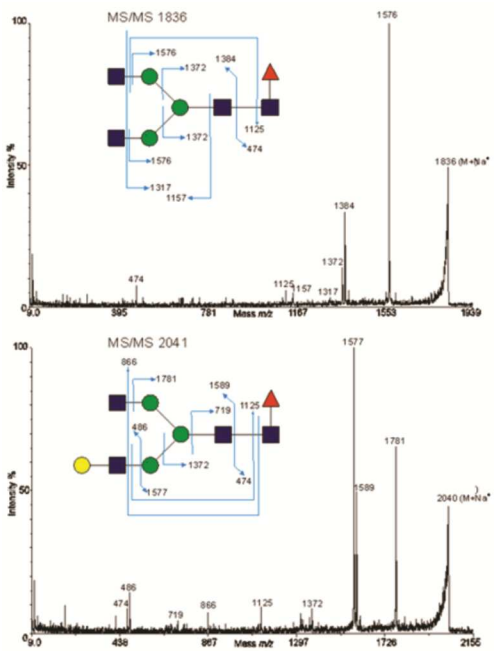
Figure 1. *N*-glycans influence the immunoglobulin recognition of a TE prepared from female *A. cantonensis* worms. C+: TE probed with positive sera of CA; O: TE treated with *O*-glycosidase; N: TE treated with *N*-glycosidase (PNGase F); FO: TE treated with α-L-Fucosidase and *O*-glycosidase; C-: TE probed with negative sera of CA; O-, N-; OF-: TE treated with glycosidases and probed with negative sera of CA. MP: TE treated with *m*-periodate and probed with positive sera of CA; MP-: TE treated with *m*-periodate and probed with negative sera of CA.

Figure 2. MALDI-TOF spectrum of permethylated *N*-glycans in TE prepared from female *A. cantonensis* worms. *N*-glycans in the TE were enriched by using different biotinylated lectin columns. Bound glycans were released from the tryptic glycopeptides by digestion with PNGase F and permethylated prior to analysis.

Figure 3. MALDI-TOF/-TOF-MS/MS spectrum of the two major peaks, *m/z* 1836 and *m/z* 2041, that were identified in the MS analysis of the TE prepared from female *A. cantonensis* worms. The complex *N*-glycan at *m/z* 2041 included GlcNAc and LacNAc structures that could be present on either antennae.







HIGHLIGHTS

1- Female *A. cantonensis* worms antigen contains complex-type, high mannose and truncated N-glycans.

2 – *N*-glycans are fundamental for the antigenicity of 31-kDa antigen of the female *A. cantonensis* worms.

3 – The main glycan of female *A. cantonensis* worms is a complex-type *N*-glycans, with core α1-6 fucosylated and truncated antennas.